BESCHWERDEKAMMERN DES EUROPÄISCHEN PATENTAMTS

BOARDS OF APPEAL OF THE EUROPEAN PATENT OFFICE

CHAMBRES DE RECOURS DE L'OFFICE EUROPEEN DES BREVETS

Internal distribution code:

(A) [ ] Publication in OJ
(B) [ ] To Chairmen and Members
(C) [ ] To Chairmen
(D) [X] No distribution

Datasheet for the decision of 29 September 2010

Case Number: T 0851/09 - 3.3.08

Application Number: 99958782.7

Publication Number: 1127125

IPC: C12N 15/12

Language of the proceedings: EN

Title of invention:
Methods for transforming plants to express Bacillus thuringiensis delta-endotoxins

Applicant:
Monsanto Technology LLC

Headword:
Delta endotoxin/MONSANTO

Relevant legal provisions:
EPC Art. 56

Keyword:
"Inventive step (yes)"

Decisions cited:
-

Catchword:
-

Europäisches Patentamt | European Patent Office | Office européen des brevets

Beschwerdekammern | Boards of Appeal | Chambres de recours

Case Number: T 0851/09 **-** 3.3.08

D E C I S I O N
of the Technical Board of Appeal 3.3.08
of 29 September 2010

Appellant: Monsanto Technology LLC
800 North Lindbergh Boulevard
St. Louis, Missouri 63167 (US)

Representative: Helbing, J.
von Kreisler Selting Werner
Deichmannhaus am Dom
Bahnhofsvorplatz 1
D-50667 Köln (DE)

Decision under appeal: **Decision of the Examining Division of the European Patent Office posted 20 November 2008 refusing European patent application No. 99958782.7 pursuant to Article 97(2) EPC.**

Composition of the Board:

Chairman: C. Heath
Members: L. Galligani
P. Julià

Summary of Facts and Submissions

I. The appeal was lodged by the applicant against the decision of the examining division dated 20 November 2008 whereby the European patent application No. 99 958 782.7 published as WO 00/26371 (hereinafter referred to as "the application as filed") was refused on the basis of Article 97(2) EPC. Basis for the refusal was the set of claims 1 to 25 filed on 10 October 2008 of which claims 1 and 19 were considered to lack an inventive step (Article 56 EPC). The claims in question read as follows:

"1. A plant or plant cell having a Cry2Ab ***Bacillus thuringiensis*** δ-endotoxin protein localized in a subcellular organelle or compartment.

"19. A nucleic acid sequence comprising a promoter operably linked to a first polynucleotide sequence encoding a plastid transit peptide, which is linked in frame to a second polynucleotide sequence encoding a Cry2Ab ***Bacillus thuringiensis*** δ-endotoxin protein, wherein expression of said nucleic acid sequence by a plant cell produces a fusion protein comprising an amino-terminal plastid transit peptide covalently linked to said δ-endotoxin protein, and wherein said fusion protein functions to localize said δ-endotoxin protein to a subcellular organelle or compartment."

II. The reasoning which led the examining division to the refusal was briefly the following:
The closest prior art document D1 (see infra) disclosed a nucleic acid sequence encoding a Cry1Ac ***Bacillus thuringiensis*** δ-endotoxin protein which was linked to a

promoter and to a chloroplast transit sequence, said endotoxin being active against lepidopteran insect pest. Claim 19 differed from the known construct in that it referred to polynucleotide encoding a Cry2Ab *Bacillus thuringiensis* δ-endotoxin protein. The latter was known from prior art document D2 (see infra) which described two *Bacillus thuringiensis* δ-endotoxin proteins, namely CryB1 and CryB2, the latter being 100% identical over a 633 amino acid overlap to Cry2Ab. In consideration of the fact that:
i) D1 did not report any problems with the Cry1Ac protein in transgenic plants;
ii) there was no particular prejudice in the art against the use in plants of a specific endotoxin with lepidopteran activity;
iii) the present application did not report any evidence of a superior property or effect of the Cry2Ab protein, and
iv) the latter was for the skilled person just one of the limited number of equally likely alternatives (cf. document D6, infra),
no inventive step could be acknowledged for the subject matter of claim 19 and, for the same reasons, also for that of claim 1.

III. The appellant filed with the statement of grounds of appeal an amended set of claims (claims 1 to 23) wherein claim 1 read as follows:

"1. A plant or plant cell comprising a synthetic nucleic acid sequence comprising a plant functional promoter sequence operably linked to a first polynucleotide sequence encoding a plastid transit peptide, which is linked in frame to a second

polynucleotide sequence encoding said Cry2Ab *Bacillus thuringiensis* δ-endotoxin protein, wherein said plastid transit peptide functions to localize expression of said δ-endotoxin protein to a subcellular organelle or compartment of the plant or plant cell."

IV. The examining division did not rectify its decision and the appeal was referred to this board of appeal under Article 109(2) EPC.

V. In reply to the communication of the board dated 15 December 2009 with some preliminary considerations on the inventive step issue, the appellant filed a new set of claims with some minor amendments. Then, in reply to the board's communication dated 31 May 2010 annexed to the summons to oral proceedings, the appellant filed a further amended set of claims (1 to 24).

VI. Oral proceedings took place on 29 September 2010. During the oral proceedings, the pending request was replaced by another request (claims 1 to 21) wherein claims 1 and 15 read as follows:

"1. A plant cell comprising a synthetic nucleic acid sequence comprising a plant functional promoter sequence operably linked to a first polynucleotide sequence encoding a plastid transit peptide, which is linked in frame to a second polynucleotide sequence encoding a Cry2Ab *Bacillus thuringiensis* δ-endotoxin protein, wherein said plastid transit peptide functions to localize expression of said δ-endotoxin protein to a subcellular organelle or compartment of the plant cell.

15. A transgenic plant genetically modified to contain, localize and express the synthetic nucleic acid sequence according to any one of the preceding claims to a subcellular organelle or compartment of cells of said plant."

Dependent claims 2 to 14 concerned particular embodiments of the plant cells according to claim 1.
Dependent claims 16 and 17 were directed to the plant of claim 15, said plant being respectively a monocotyledonous or a dicotyledonous plant.
Independent claim 18 was directed to seed or progeny of the plant of claims 15 to 17.
Independent claim 19 and dependent claims 20 to 21 were directed to a nucleic acid sequence according to the scheme outlined in claim 1.

VII. The following documents are referred to in the present decision:

(D1) E.Y. Wong et al., Plant Molecular Biology, Vol. 20, 1992, pages 81 to 93;

(D2) W. R. Widner and H.R. Whiteley, J.Bacteriol., Vol. 171, No. 2, February 1989, pages 965 to 974;

(D6) N. Crickmore et al., Microbiology and Molecular Biology Reviews, Vol. 62, No.3, September 1998, pages 807 to 813.

VIII. The position of the appellant in support of its request for revision of the decision under appeal can be summarised as follows:

The disclosure in document D1 of a nucleic acid construct comprising a sequence encoding a Cry1Ac *Bacillus thuringiensis* δ-endotoxin protein in conjunction with a promoter and a sequence encoding a chloroplast transit peptide and its use for targeting the expression of the endotoxin in plant cells and transgenic plants was indeed to be seen as the closest prior art. This prior art was also acknowledged in the application as filed (cf. page 21, lines 6 to 15). However, the technical problem underlying the present invention could not simply be seen in finding an alternative to such a construct, but rather in finding a system giving higher rates of expression of the endotoxin into the cells/plants thereby overcoming the problem of development of insect resistance. In fact, as outlined in the introductory part of the application, a serious problem of the known prior art systems was the low level of expression which resulted in the development of insect resistance and thus poor results. The experimental evidence in the application (see Tables 1 to 4) showed that the expression system according to the invention achieved high rates of expression and thus also a delay or elimination of the development of resistance to the endotoxin. The system proposed could not be considered obvious for the skilled person because, although it might have been obvious to try to find an alternative system, the selection of the Cry2Ab *Bacillus thuringiensis* δ-endotoxin protein among the huge number of possible available candidates (see document D6) was not an immediate option for the skilled person. In fact, faced with the problem of finding an improved system, the skilled person would have resorted first to a simple expression system such as cytoplasmatic expression in

order to screen for possible candidate endotoxins before trying the boosting in chloroplasts. In such a system, as shown by the present patent application, Cry2Aa (corresponding to CryB1 of document D2) performed better than Cry2Ab (corresponding to CryB2 of document D2), compare e.g. Examples 4 and 5, Tables 11 and 12. Thus, under the hypothesis that the skilled person would have directed his/her attention to the two endotoxins of document D2 (an assumption which, in view of the large list of possible candidate endotoxins of different structures and insecticidal spectrum, implied already some hindsight), he or she would have focused on Cry2Aa rather than on Cry2Ab, and would have then found in a targeted system that, as shown in the present application, a decreased expression rate and increased phytotoxicity were obtained using Cry2Aa (cf. Example 6). This finding would have disqualified ***a priori*** also the other candidate Cry2Ab, in view of their structural similarity. Thus, the finding by the present inventors that Cry2Ab actually performs much better in a targeted than in an untargeted system, and that there it performs much better than Cry2Aa (cf. Examples 1 to 6) was quite unexpected and demonstrated also that, contrary to the assumption of the examining division, not all endotoxins were alike. This justified the acknowledgement of an inventive step.

IX. The appellant requested that the decision under appeal be set aside and that the patent be granted on the basis of the request as filed in the oral proceedings, with claims 1 to 21.

Reasons for the decision

1. The new set of claims filed during the oral proceedings does not raise any formal issues under Article 123(2) EPC and Article 84 EPC as their subject-matter is supported by the application as originally filed and is clearly defined in terms of the technical features of the invention. With reference to the published version of the application as filed, the various embodiments of the claimed plant cell, the nucleic acid which the cell comprises as well as the transgenic plant modified to contain, localize and express it are disclosed *inter alia* on pages 3 to 6, 9 to 11 of the description and in the examples. The nucleic acid construct which is introduced into the plant cell (either monocotyledonous or dicotyledonous) is clearly described in its components parts and their arrangement. This allows a clear recognition of the scope of the claim also in respect of the generic plant cell and the generic transgenic plant (seed and progeny thereof) containing such a construct which are required to localize it and express it in a subcellular organelle or compartment.

2. The examining division rejected the application on grounds of lack of an inventive step, no objections being raised in respect of either sufficiency of disclosure and novelty. The board has no objections of its own in respect of these latter issues. Thus, the only pending substantive issue is whether the claimed subject-matter would have been obvious for a skilled person.

3. Admittedly, systems for targeting a *Bacillus thuringiensis* δ-endotoxin to the chloroplasts of a

plant cell based on the arrangement outlined in claim 1 were known in the art. Prior art document D1 described such a construct based on the *Bacillus thuringiensis* δ-endotoxin CryIA(c) and proposed its use in the development of insect-resistant crops. This document was in fact identified as the closest prior art. The claimed subject-matter differs from this disclosure in that it is based on the *Bacillus thuringiensis* δ-endotoxin Cry2Ab, known in the art (cf. document D2) also under the name CryB2 and listed in Table I of document D6 (cf. reference 123 therein).

4. The above technical circumstances have led the examining division to the conclusion that the skilled person, faced with the problem of finding a system alternative to that of document D1, would have in an obvious manner arrived at the choice of CryB2 of document D2 (= Cry2Ab), as this was just one of the limited number of equally likely alternatives listed in document D6. In the examining division's view this conclusion was further supported by the fact that neither particular problems nor prejudices were known in the art in relation to the use of a specific endotoxin with lepidopteran activity, and that the present application did not report any superior property or effect linked to the choice of Cry2Ab (cf. Section II supra).

5. As observed by the appellant during the oral proceedings, the above reasoning looks only *prima facie* convincing. It fails, however, to take into account the experimental evidence provided by the application under scrutiny, which, although not making a direct comparison between the prior art system and the one

claimed, reports a direct comparison between the targeted and untargeted expression of the two possible candidate endotoxins of document D2. The said evidence is of relevance as it confirms two assertions made by the appellant, namely i) that not all endotoxins can ***a priori*** be expected to behave alike under targeted conditions; and ii) that the achievement of a sufficiently high level of expression of the endotoxin was indeed part of the problem to be solved. These two factors cannot be overlooked in a "real life" analysis of inventive step.

6. Moreover, in trying to imagine the mental process of the skilled person faced with a given technical problem, the chronology of the development in the art should also not be disregarded. In the present case, the starting point in the reasoning is document D1 published in 1992. This document contains no specific reference to document D2 published in 1989. Thus, ***a priori*** the skilled person, faced with the problem of developing a further (improved) system departing from document D1, would have not necessarily directed his/her attention specifically to the two endotoxins of document D2, but would have had to choose among the much broader range of possibilities offered in the art, cf. Table 1 of document D6 published in September 1998. Would the skilled person have had any reason to choose the Cry2Ab from this vast number of possible candidates? The answer is certainly no. Would a choice of an endotoxin be perfectly equivalent to the choice of another one? The answer is also no, because the fact that all the listed endotoxins display insecticidal activity does not ***per se*** justify the assumption that they would all behave alike in a targeted system such

as that described in document D1, due to differences in their structure and mode of action. The experimental data provided in the patent application confirm this, because, in spite of their high degree of identity, Cry2Aa and Cry2Ab are shown to have opposite effects.

7. Thus, when carrying out a "real life" analysis of the inventive issue, it has to be acknowledged that the results reported in the patent application would have been quite surprising for the skilled person who in proceeding experimentally as indicated by the appellant (see Section VIII supra), i.e. resorting first to a simple expression system such as cytoplasmatic expression in order to screen for possible candidate endotoxins before trying the boosting in chloroplasts and observing that in such a system Cry2Aa (corresponding to CryB1 of document D2) performed better than Cry2Ab (corresponding to CryB2 of document D2), would not have expected the latter to provide such good results in a targeted system.

8. The above reasoning justifies in the board's judgement the acknowledgement of an inventive step for the subject-matter of the present claims which are all focused on the same specific targeted system.

Order

For these reasons it is decided that:

1. The decision under appeal is set aside.

2. The case is remitted back to the first instance with the order to grant a patent based on the set of claims filed in the oral proceedings, and a description and drawings to be adapted thereto.

The Registrar: The Chairman:

A. Wolinski C. Heath